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Gene Action, Heterotic Patterns, and Inter-Trait Relationships of Early Maturing Pro-Vitamin a Maize Inbred Lines and Performance of Testcrosses Under Contrasting Environments

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Abstract: Vitamin A deficiency is the leading cause of night blindness, total blindness, maternal and childhood mortality in developing countries. Drought, low soil nitrogen and *Striga hermonthica* parasitism are major constraints to maize production in sub-Saharan Africa (SSA). Thus, the development of multiple stress tolerant maize varieties with elevated levels of PVA is an economically feasible approach to simultaneously tackle malnutrition and food insecurity in SSA. The objectives of this study were to determine the gene action modulating the inheritance of grain yield and other traits, group the inbred lines, investigate inter-trait relationships among grain yield and other traits and assess the performance and stability of single-cross hybrids derived from a set of inbred lines under stress and non-stress environments. One hundred and ninety diallel crosses plus six hybrid checks were evaluated under managed drought at Ikenne during the 2016/17 and 2017/18 dry seasons, low soil N conditions at Mokwa and Ile-Ife, *Striga* infestation at Abuja and Mokwa, and optimal management conditions at Ikenne, Kadawa, Abuja, Bagauda and Mokwa during the 2016 and 2017 growing seasons. Both additive and non-additive gene actions were prominent in the inheritance of grain yield and other measured traits under stress and optimal management conditions. However, additive gene action was preponderant over the non-additive. The PVA inbreds were classified into three heterotic groups with TZEI 25 and TZEIOR 164 identified as inbred testers for heterotic groups 2 and 3, respectively. Plant and ear heights, ears per plant, plant and ear aspects were identified as reliable secondary traits for genetic enhancement of grain yield under both stress and non-stress conditions. Hybrids TZEIOR 4 x TZEIOR 158 and TZEIOR 119 × TZEIOR 158 were outstanding in performance and should be tested extensively for possible commercialization to combat malnutrition and food insecurity in SSA.

Keywords: gene action; heterotic patterns; testcrosses; *Zea mays* L.

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1. Introduction

Maize (*Zea mays*) is the most cultivated cereal crop in sub-Saharan Africa (SSA) and has the potential to combat the challenges of food insecurity and malnutrition presently facing the region. The crop is cultivated on about 100 million hectares across the developing world and it is an important source of at least 30% of dietary calories for about 310 million people [1]. Due to increasing population and wider acceptability, the demand for maize in the developing world is projected to double by 2050. Therefore, there is a dire need for increased production of maize to meet this demand [2,3]. Maize is largely cultivated for its starchy endosperm which contributes a large portion of human energy intake. However, normal endosperm maize is deficient in vitamin A, which unfortunately the human body is unable to synthesize but must be sourced from external food supplements. Maize as a sole source of food exposes human populations in sub-Saharan

Africa (SSA) to the risk of health challenges associated with vitamin A deficiency. Therefore, maize consumers in SSA rely on the dietary sources from plant tissues to meet its vitamin A requirements. Vitamin A deficiency is responsible for several health disorders including night blindness, total blindness and it is the leading cause of maternal and childhood mortality in developing countries [4], Development and commercialization of high yielding maize, fortified with vitamin A, is a realizable strategy for combating both hunger and health challenges related to malnutrition in SSA. Additionally, recurrent drought, low soil nitrogen and *Striga hermonthica* parasitism are major limiting factors to increased maize production and productivity in SSA. Yield loss due to recurrent drought is considered one of the greatest challenges facing agricultural production in SSA [5]. The occurrence and severity of drought have been projected to increase in all regions of the world leading to a downward trend in maize production. The highest yield reduction is recorded when drought occurs at the reproductive and grain filling periods, which often leads to reduced ear and kernel sizes and increased anthesis-silking interval in maize [6]. Badu-Apraku et al., [7] reported 44% yield reduction in some selected early-maturing maize varieties under moisture deficit conditions when the yield of the varieties was compared to their mean yield under well-watered environments. *Striga hermonthica* parasitism is another leading cause of yield loss in maize in the savanna of West and Central Africa (WCA) [8]. *S. hermonthica* is a parasitic weed which attacks maize, guinea corn and other cereal crops, and it is the most economically important biotic stress factor that limits maize grain yield in SSA, where majority of the diets are cereal based [9]. The damage caused by *Striga* is widespread, affecting the livelihoods of over 100 million Africans through reduction in grain yield [10]. The high incidence of the parasite in the savanna and mid-altitude agro-ecologies, areas with the highest maize yield potentials, severely limits the maize production capacity of SSA [10]. Kim [11] reported that under controlled environment conditions, *Striga* infestation causes an average yield reduction of 67% with a range of 41–91% depending on the severity of infestation and host plant susceptibility levels. Several control measures are available; however, none is capable of ensuring total control of the parasitic weed, necessitating an integrated management approach [10]. Genetic control via use of improved cultivars possessing *Striga* resistance/tolerance alleles has been proposed as a reliable strategy for minimizing *Striga* damage in SSA [12,13]. In a study conducted to compare the yield losses due to drought stress and *Striga* parasitism, [14] reported yield reductions of 53% and 42% under drought conditions and *Striga* infestation, respectively. Several factors, such as continuous cropping, declining soil fertility, short fallow as well as expansion of production into marginal lands have contributed immensely to the prevalence of *Striga* parasitism [10,15,16]. Low soil nitrogen is another abiotic stress that constrains the maize production capacity of WCA. In most developing countries, peasant farmers produce maize under depleted soil-nitrogen conditions due to lack of access to fertilizer which may be attributed to non-availability, and high cost and non-availability of loan facilities to farmers [17]. Reduction in grain yield due to low N is estimated to vary between 10 to 50% [18].

The IITA Maize Program has used plant breeding strategies such as backcrossing, inbreeding, hybridization, and the S_1 recurrent selection to develop early maturing *Striga* resistant, drought and low-N tolerant inbred lines with high Pro-vitamin A levels (PVA). However, information on the extent and patterns of genetic diversity among germplasm lines is critical to the success of a hybrid development program. This helps in the identification and selection of parents for the development of superior hybrids as well as identification of sources of favorable alleles for introgressive hybridization [9]. Knowledge of the mode of gene action modulating grain yield and other traits of inbred lines under stress conditions is critical for identifying good combiners and promising hybrids, and for designing appropriate strategies for developing multiple stress-tolerant hybrids. This information could be obtained through combining ability studies. However, results of several combining ability studies conducted are inconsistent. The

inconsistencies observed in the findings call for further studies to clarify the contradictions about the type of gene action involved in the inheritance of grain yield and other traits under stress conditions. Additionally, it is important to assess combining ability and heterotic patterns of the several early maturing PVA inbreds recently developed in the IITA-MIP and to classify them into heterotic groups to exploit their genetic potential in hybrid combinations. Combining ability analysis is a powerful tool for identifying genotypes for crosses in order to fully exploit hybrid vigor and identify outstanding hybrids for direct use or for further breeding [19]. The genetic improvement of grain yield and other desirable traits depend on the nature and magnitude of genetic variability and interactions involved in the inheritance of the traits and can be estimated using diallel cross technique [20]. Diallel mating design has been effective for determining the gene action controlling the inheritance of grain yield and other important traits, estimating the general combining ability (GCA) of parental lines, identifying superior inbred parents for hybrid or synthetic cultivar development, classifying inbred lines into heterotic groups and identifying appropriate testers for use in breeding programs in SSA [21–24]. The present study was carried out to (i) determine the gene action modulating inheritance of grain yield and other traits (ii) estimate general and specific combining abilities of 20 PVA early maturing inbred line and classify them into heterotic groups (iii) assess the performance and stability of the single-cross hybrids under stress and non-stress conditions and (iv) investigate interrelationships among grain yield and other traits under stress and non-stress conditions.

2. Materials and Methods

2.1. Development of PVA Maize Inbred Lines

The broad based early maturing drought and *Striga* resistant yellow/orange variety, 2004 TZE-Y Pop DT STR C4, was crossed to a source of high provitamin A [Syn –Y-STR-34-1-1-1-1-2-1-B-B-B-B-B/NC354/SYN-Y-STR-34-1-1-1 (OR1) intermediate maturing population from the IITA Maize Improvement Program to introgress the genes for high beta-carotene into the variety. Subsequently, the top-cross hybrid was backcrossed to the recurrent population to recover earliness, resulting in BC_1F_1 progenies. This was followed by a cycle of backcrossing to 2004 TZE-Y Pop DT STR C_4 to recover earliness. The kernels of BC_1F_1 with deep orange color were selected and recombined to obtain the early provitamin A variety 2009 TZE-OR_1 STR. This was followed by selfing of 2009 TZE-OR_1 STR to obtain S_1 lines which were advanced through repeated selfing to the S_6 stage to develop 155 early provitamin A inbred lines. The kernels of the BC_1F_1 of each material with deep orange color were selected and self-pollinated for two cycles for advancement to the BC_1F_3 stage. Furthermore, BC_1F_3 lines with the deep orange color were selected and recombined to form the early Provitamin A variety, 2009 TZE-OR_1 STR (Figure 1). Due to fund limitations the provitamin A variety could not be screened for beta-carotene content. However, the variety was included in the regional uniform variety trials under multiple contrasting environments in 2010 and several of them showed outstanding performance. Additionally, the early varieties 2009 TZE-OR1 DT STR out-yielded the commercial OPV check, TZE Comp 3 and DT C_2 F_2 by 12 and 11% across eight contrasting environments in WCA [25]. Following the development of the PVA inbred lines, genetic studies were conducted to determine the combining ability of the lines and to identify inbred testers. Several outstanding inbred lines identified were crossed among themselves to develop bi-parental crosses including (TZEI 17 x TZEI 11) from which additional inbred lines were developed through pedigree selection [26]. Furthermore, selected inbred testers were crossed to the OPVs including 2009 TZE-OR1 STR for development of top-cross hybrids. The 20 early maturing pro-vitamin A inbred lines used for the present study included lines extracted from (TZEI 17 x TZEI 11) and 2009 TZE OR1 DT STR. Selection of the inbred lines for the present study was based on the evaluations conducted under managed

moisture stress at Ikenne as well as under *Striga*-infested conditions at Mokwa and Abuja in 2014 and 2015.

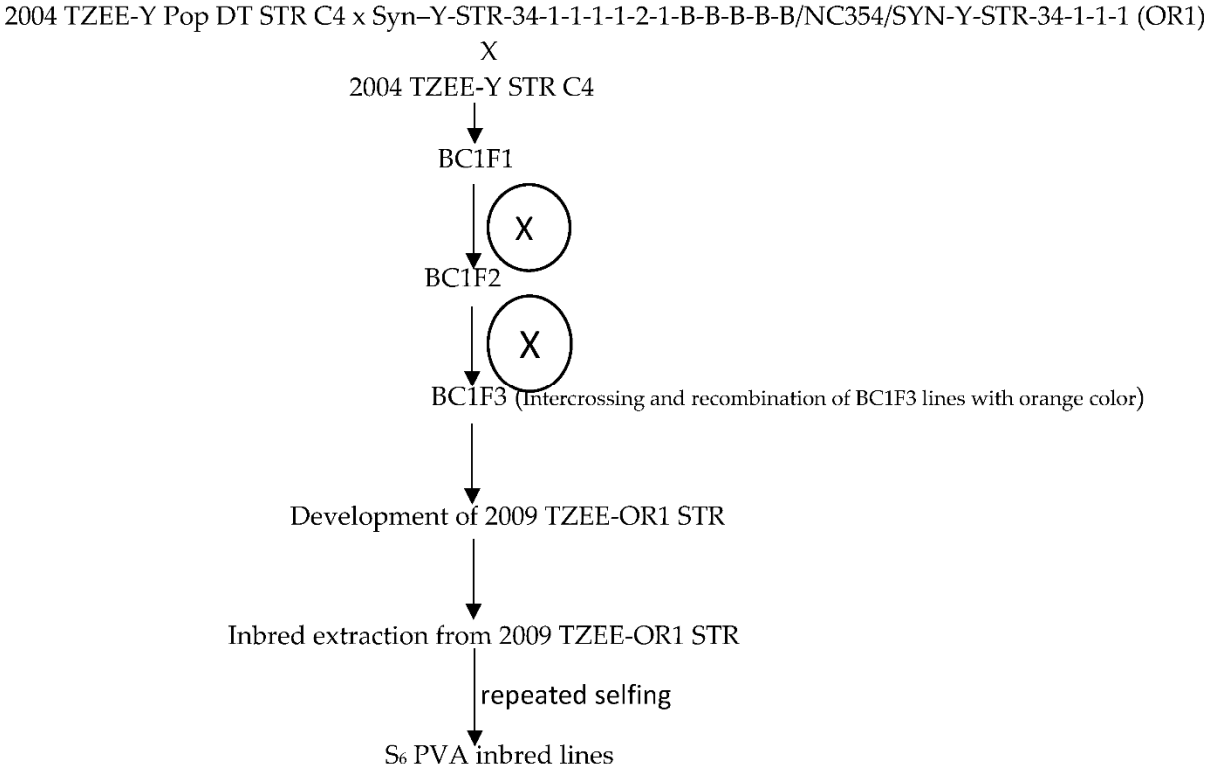


Figure 1. Extraction of the PVA inbred lines derived from the source population 2009 TZEE-OR1 STR.

2.2. Generation of the Diallel Crosses

The 20 PVA inbred lines selected for this study were planted in the breeding nursery at IITA headquarters, Ibadan, during the growing season of 2016. All the inbred lines were mated in a diallel to produce 190 F_1 hybrids excluding reciprocals. The 190 hybrids along with six checks (three yellow normal-endosperm early maturing single-cross hybrids and three top-cross hybrids were evaluated in the present study.

2.3. Field Evaluations

The hybrids (190 single-cross hybrids plus 6 checks) were evaluated between 2016 and 2017. The trial was evaluated under managed moisture stress at Ikenne (6°53′ N, 30°42′ E, 60 m altitude) during the dry seasons of 2016/2017 and 2017/2018. The plants were irrigated using sprinkler irrigation system that delivered 17 mm of water each week for the first three weeks. Drought stress was imposed by withdrawing irrigation at 25 days after planting (DAP) until maturity, thus making the maize plants rely on water stored in the soil for growth and development. The soil at the IITA experimental station at Ikenne is eutric nitrosol [27], with high water holding capacity. The experiment was planted on a flat and uniform field. Fertilizer was applied at planting at the rate of 60 kg N/ha, 60 kg P/ha and 60 kg K/ha, with an additional 60 kg N/ha applied as top-dress at 4 WAP.

Evaluations under *Striga* infestation was carried out at Mokwa (9°18′ N, 5°4′ E, 457 m altitude, 1100 mm rainfall) in 2016 and 2017 and Abuja (9°16′ N, 7°20′ E, 300 m altitude, 1500 mm rainfall) in 2017 in the southern Guinea savanna of Nigeria. *Striga* infestation was in accordance with the method of [11] and [28]. *Striga hermonthica* seeds collected from sorghum (*Sorghum bicolor*) fields and stored for at least 6 months to break the seed dormancy were used for the present study. Ethylene gas was injected into the soil 2 weeks before artificial infestation to stimulate suicidal germination of existing *Striga* seed in the

field. Application of fertilizer was delayed until about 21–25 DAP when 30 kg/ha each of N, P and K were applied as NPK 15-15-15. The delay was necessary to stimulate the production of strigolactones to cause suicidal germination of the *Striga* seeds in the soil. Weeds other than *Striga* were controlled by manual weeding.

Under low-N, the single-cross hybrids plus checks were planted at Mokwa during the major growing season of 2016 and 2017. The soil at Mokwa is luvisol [27] with 0.27%, 0.04% and 0.48% by volume of organic carbon, organic nitrogen and phosphorus content, respectively. Maize was planted on the experimental field for several years and the biomass was removed after each harvest to deplete the N in the soil. Soil samples were taken, and the N content of the soil was determined at IITA Analytical Laboratory Services, Ibadan, using the Kjeldahl digestion and colorimetric methods [29]. Fertilizer was applied to bring the total available N to 30 kg N/ha at 2 WAP. Single superphosphate (P_2O_5) and muriate of potash (K_2O) were applied to the low-N blocks at the rate of 60 kg/ha.

Under optimal growing conditions, the single-cross hybrids plus the checks were evaluated at Ikenne during the growing season of 2016 and 2017, Mokwa and Kadawa in 2016, Abuja and Bagauda in 2017. Water and nitrogen were not limiting under the optimal growing conditions, and weeds were effectively controlled. The trials received 60 kg/ha each of N, P and K at 2 WAP with an additional 30 kg N/ha top-dressed at 4 WAP. A 14 × 14 randomized incomplete-block design with two replications was used in each of the trials. The experimental unit was single-row plots, 3 m long with spacing of 0.75 m between rows and 0.40 m between plants within a row in all the trials. Three seeds were planted per hill, and the seedlings were thinned to two seeds per hill at about 2 weeks after emergence to give a final population density of about 66,666 plants ha^{-1}. All experiments except those under *Striga* infestation were kept weed-free with the application of Atrazine and Gramoxone at 5 L/ha as pre- and post-emergence herbicides, respectively.

2.4. Kernel Samples Production for Quantification of PVA Contents

Seed samples used for the carotenoid analyses in the present study were produced as described by [30] by self-pollinating the hybrids and 20 PVA inbred lines to produce kernel samples for analysis of the PVA contents. The 54 hybrids were selected from the 196 derived hybrids based on GY performance. The 20 inbred lines were parents involved in generating the 54 hybrids using the diallel mating arrangement. The genotypes were planted under well-watered conditions at IITA-Ibadan (7°28′11.99″ N, 3°53′2.88″ E, altitude 190 m) and Ikenne in 2018 using 1 m long single rows with 0.75 m between rows and 0.20 m within rows. One plant per hill was established to provide five plants per plot. Maize grain samples of hybrids and inbred lines were produced by self-pollination of individual plants in each plot. At physiological maturity, the self-pollinated ears of the inbred lines and hybrids of each location were harvested per plot, dried under ambient temperature, and shelled [31]. The seed samples were stored in the long-term storage facility of IITA at 4 °C. Seed samples of the 20 inbred lines used for the diallel crosses along with the top yielding 13 PVA hybrids plus two checks obtained from compositing grains harvested separately from the inbred lines and hybrid trials of the two locations were drawn from the long-term storage and the carotenoids were extracted and quantified at the Food and Nutritional Laboratory of IITA, Ibadan, Nigeria. The High-Performance Liquid Chromatography (HPLC) method, based on the extraction protocol described by [32], was employed for the carotenoid analysis. The five carotenoids, β -carotene (cis and trans isomers), α-carotene, β-cryptoxanthin, zeaxanthin, and lutein were determined based on calibrations using external standards. Total carotenoids were computed as the sum of concentrations of α-carotene, β-carotene, lutein, zeaxanthin and β-cryptoxanthin. PVA was computed as the sum of β-carotene, and half of each of β-cryptoxanthin and α-carotene contents, because β-cryptoxanthin and α-carotene contribute about 50% of the β-carotene as PVA according to the [33]. Two independent measurements were taken to

represent each sample. In addition to the PVA levels of the hybrids (HPVA) determined by chemical analysis, those of the mid-parent (MP) were calculated as the average of the sum of PVA levels of parental inbred lines (Table 1) involved in hybrid development.

Table 1. Characteristics and parentage of early-maturing maize inbred lines used for the diallel study.

S/N	Pedigree	Parentage	Reaction to Stress		Provitamin A Content (μg/g)
			Drought	*Striga* Hermonthica	
1	TZEIOR 2	2009 TZE OR1 DT STR	Tolerant	Susceptible	4.91
2	TZEIOR 4	2010 TZE OR1 DT STR	Tolerant	Tolerant	6.45
3	TZEIOR 6	2011 TZE OR1 DT STR	Tolerant	Tolerant	6.34
4	TZEIOR 30	2012 TZE OR1 DT STR	Tolerant	Susceptible	6.30
5	TZEIOR 52	2013 TZE OR1 DT STR	Tolerant	Tolerant	7.96
6	TZEIOR 62	2014 TZE OR1 DT STR	Tolerant	Tolerant	5.32
7	TZEIOR 68	2015 TZE OR1 DT STR	Tolerant	Tolerant	8.18
8	TZEIOR 73	2016 TZE OR1 DT STR	Tolerant	Tolerant	7.62
9	TZEIOR 79	2017 TZE OR1 DT STR	Tolerant	Susceptible	5.48
10	TZEIOR 117	2018 TZE OR1 DT STR	Tolerant	Susceptible	4.19
11	TZEIOR 119	2019 TZE OR1 DT STR	Tolerant	Susceptible	3.61
12	TZEIOR 124	2020 TZE OR1 DT STR	Tolerant	Susceptible	3.28
13	TZEIOR 125	2021 TZE OR1 DT STR	Tolerant	Tolerant	3.75
14	TZEIOR 157	(TZEI 17 × TZEI 11)	Susceptible	Tolerant	4.42
15	TZEIOR 158	(TZEI 17 × TZEI 11)	Tolerant	Susceptible	2.65
16	TZEIOR 163	(TZEI 17 × TZEI 11)	Tolerant	Tolerant	2.95
17	TZEIOR 164	(TZEI 17 × TZEI 11)	Tolerant	Susceptible	7.40
18	TZEIOR 165	(TZEI 17 × TZEI 11)	Susceptible	Tolerant	4.50
19	TZEI 25	TZE-Y Pop STR Co	Tolerant	Tolerant	5.30
20	TZEI 129	TZE-Y Pop STR Co	Tolerant	Tolerant	8.16

2.5. Data Collection

In all the environments, data were recorded for days to silking and days to anthesis as the number of days from planting to when 50% of the plants in the plot had extruded silks and had shed pollen. The anthesis-silking interval (ASI) was calculated as the difference between the number of days to silking and number of days to anthesis. Plant height was measured as the distance from the base of the plant to the height of the first tassel branch and ear height as the distance from the base of the plant to the node bearing the upper ear. Root lodging (the percentage of plants leaning more than 30^0 from the vertical), and stalk lodging (percentage of plant broken at or below the ear node) were recorded. For drought and low-N experiments, stay-green characteristic was measured at 70 DAP on a scale of 1 (almost all leaves green) to 9 (virtually all leaves dead). Plant aspect was scored on a scale of 1 (excellent plant type) to 9 (poor plant type). Husk cover was scored on the scale of 1 (husk tightly arranged and extended beyond the tip of the ear) to 5 (ear tips exposed). Ear aspect was measured on the scale of 1 (clean, uniform, large and well-filled ears) to 9 (ears with undesirable features). Number of ears per plant (EPP) was calculated by dividing the total number of ears harvested per plot by number of plants in the plot. Percentage moisture was determined from shelled harvested ears. For trials under drought and low-N, harvested ears were shelled and weighed, grain yield in kg/ha was computed from the grain weight, adjusted to 15% moisture. For the optimal and *Striga* experiments, a shelling percentage of 80% was assumed for all hybrids and grain weight (obtained from ears weighed and converted to kg/ha) was adjusted to 15% moisture. The data recorded under *Striga* experiments were the same as those obtained under low-N except that no data was recorded on plant aspect and stay-green characteristic. *Striga* damage [13] and number of emerged *Striga* plants at 8 and 10 WAP (50 and 70 DAP) in

the *Striga* infested plots. *Striga* damage ratings were scored on the scale of 1 (no damage, indicating normal plant growth) to 9 (complete collapse or death of the maize plant i.e highly susceptible). Even though data were collected on several traits, only those on the most important traits in the studies were presented.

2.6. Statistical Analysis

Analysis of variance was conducted on data collected across locations and years under each research condition with PROC GLM in SAS using RANDOM statement with the test option [34]. Furthermore, combined ANOVA were carried out across the 13 research environments with the sources of variation such as environment, replicates within environments and block within replicate × environment considered as random factors while hybrids (190 plus six checks) were regarded as fixed factors. The general combining ability (GCA) effects of the parents and the specific combining ability (SCA) effects of the crosses, as well as their mean squares in each environment and across research environments were estimated for the 190 diallel crosses following Griffing's method 4 model 1 (fixed model) ([35] and the DIALLEL-SAS program developed by [36] adapted to SAS software version 9.3 [34]. The GCA and SCA effects were tested for significance using t-test. The relative importance of the GCA was investigated using the method of [37] as modified by [38].

Inbred lines were assigned into heterotic groups based on GCA of multiple traits (HGCAMT) grouping method proposed by [39]. Grouping was achieved by standardizing the GCA effects of each trait that had significant mean squares for G under each study condition to minimize the effects of different scales for the traits. The statistical model used was:

$$Y = \sum_{i=1}^{n} \left(\frac{(Y_i - \dot{Y}_i)}{s} \right) + \varepsilon_{ij}$$

where Y is HGCAMT, which is the genetic value measuring relationship among genotypes based on the GCA of multiple traits i to n; Y_i is the individual GCA effects of genotypes for trait I, $\dot{Y}$ is the mean of the GCA effects across genotypes for traits I, s is the standard deviation of the GCA effects of the trait I and ε_{ij} is the residual of the model associated with the combination of the inbred i and trait j.

The 20 inbred lines were then classified into heterotic groups based on the Euclidean distance generated from the HGCAMT. Ward's minimum variance cluster analysis based on GCA effects was used to assign the inbred lines to heterotic groups across environments using SAS software version 9.3. The efficiency of the HGCAMT grouping method was confirmed based on the procedure proposed by [40] and modified by [39]. The 190 hybrids were divided into two major groups i.e., inter-group and within-group crosses. These two groups were subsequently divided into high yielding hybrids (Yield of group 1 with a mean grain yield ranking among the first 64); intermediate yielding hybrids (Yield group 2 with a mean grain yield between the 65th and the 127th) and low yielding hybrids (Yield group 3 with a mean grain yield between the 128th and the 190th).

The genotype-by-trait (G × T) analysis was conducted using R (software) package GEA-R (version 4.0), [41]. Since the traits were measured in different units, they were standardized (mean = 0, standard deviation = 1). The GGE biplot model equation for the G × T analysis is as follows:

$$\left. (\hat{Y}_{ij} - \mu - \beta_j) \right/ d_j = \lambda_1 g_{i1} e_{1j} + \lambda_2 g_{i2} e_{2j} + \varepsilon_{ij}$$

where $\hat{Y}_{ij}$ is the genetic value of the combination between inbred I and trait j; μ is the mean of all combinations involving trait j; β_j is the main effect of trait j; $\lambda 1$ and $\lambda 2$ are the singular values for PC1 and PC2; g_{i1} and g_{i2} are the PC1 and PC2 eigenvectors, respectively, for inbred I; e_{1j} and e_{2j} are the PC1 and PC2 eigenvectors, respectively, for trait j; d_j is the

phenotypic standard deviation (with mean zero and standard deviation of 1); and ε_{ij} is the residual of the model associated with the combination of inbred I and trait j.

The data on grain yield was subjected to GGE biplot analysis to decompose the G × E interactions as described by [42]. The GGE biplot focused on the first two principal components (PC1 and PC2) derived by subjecting the environment centered grain yield means for each location to singular value decomposition. The data were not transformed (Transform = 0), not standardize (Scale = 0), and were environment-centered (Centering = 2). This provided information on the hybrids that were suitable for the different environments and investigation of stability of hybrids in the various environments.

3. Results

Analysis of Variance and Combining Ability of Grain Yield and Other Agronomic Traits

The result of the analysis of variance across the three stresses (Table 2) showed significant mean squares for genotype (G), environment (E) and genotype by environment interactions (GEI) for grain yield and other measured traits. Partitioning the genotypic effects into GCA and SCA components revealed that across the stresses, GCA and SCA mean squares were significant for grain yield and other measured traits except for SCA for ASI. Also, significant GCA and SCA mean squares were observed for PASP and STGR under drought and low soil N conditions, *Striga* damage at 8 and 10 WAP, and emerged *Striga* plants at 8 and 10 WAP in *Striga*-infested environments. However, the SCA mean squares for emerged *Striga* plants at 8 WAP and 10 WAP were not significant. Under optimal conditions, G, E and GEI showed significant mean squares for measured traits under optimal growing conditions (Table 3). Significant GCA and SCA mean squares were detected for all measured traits except husk cover. The GEI mean squares were significant for measured traits under stress conditions excluding emerged *Striga* plants at 8 WAP and 10 WAP. Additionally, significant GCA × E and SCA × E interaction mean squares were observed for measured traits under stress, optimal and across research conditions except GCA × E for days to anthesis, *Striga* damage at 8 and 10 WAP, emerged *Striga* plants at 8 and 10 WAP under striga -infested conditions, days to anthesis and silk under optimal conditions, SCA × E interaction mean squares for *Striga* damage and emerged *Striga* plants at 8 and 10 WAP under *Striga* infestation.

Significant and positive GCA effects for grain yield were observed for TZEI 25 and TZEIOR 164 across research environments (Table 4). Inbreds TZEIOR 52, TZEIOR 163 and TZEI 25 recorded a positive and significant GCA effect for grain yield across research environments. TZEIOR 163 and TZEI 25 had negative and significant GCA effects for *Striga* damage rating at 8 and 10 WAP while TZEIOR 52, TZEIOR 158 and TZEIOR 164 recorded negative and significant GCA effects for *Striga* damage at 8 WAP and 10 WAP as well as emerged *Striga* plants at 8 and 10 WAP. The ratio of GCA/SCA effects revealed a higher GCA effect than the SCA for grain yield and other measured traits. The highest and least genetic ratios were observed for ASI and grain yield, respectively, under optimal growing conditions. Additionally, the relative importance of GCA to SCA effects for grain yield was higher across stress conditions than the non-stress conditions (Figure 2).

The dendrogram constructed using the HGCAMT grouping method placed the parental inbred lines into three heterotic groups (Figure 3). Eleven inbred lines TZEIOR 125, TZEIOR 124, TZEIOR 79, TZEIOR 68, TZEIOR 73, TZEIOR 62, TZEIOR 129, TZEIOR 30, TZEIOR 4, TZEIOR 6 and TZEIOR 2 constituted heterotic group I. Inbreds TZEIOR 163, TZEIOR 158, TZEIOR 157, TZEIOR 25, and TZEIOR 52 were placed in heterotic group II while TZEIOR 165, TZEIOR 164, TZEIOR 119 and TZEIOR 117 were classified into group III. Inbred testers were identified based on high per se grain yield, significant and positive GCA for grain yield, and classification into heterotic groups. Based on these criteria, inbred lines TZEI 25 and TZEIOR 164 were identified as the best testers for heterotic groups II and III, respectively, while no tester was identified for heterotic group I.

Under stress conditions, grain yield ranged from 23 Kg ha^{-1} for TZEIOR 62 × TZEIOR 68 to 3341 Kg ha^{-1} for TZEIOR 119 × TZEIOR 158 (Table S1). The best PVA hybrid outyielded the best non-PVA hybrid check by 29% under stress conditions and the least performing hybrid check by 101%. Under optimal conditions, grain yield ranged from 480 kg/ha for TZEIOR 157 × TZEIOR 163 to 6588 kg/ha for TZEIOR 4 × TZEIOR 158 (Table S2), outyielding the best performing hybrid check by 43%, and the least performing hybrid check by 57%. Across research environments, grain yield varied between 781 kg/ha for TZEIOR 164 × TZEIOR 165 to 4300 kg/ha for TZEIOR 4 × TZEIOR 158. The yield of the top performing PVA hybrid was 24% better than the best performing single-cross commercial check (TZEI 124 × TZEI 25) and 49% better than the least performing single-cross hybrid check (TZEI 24 × TZEI 17) across the research environments (data not shown).

The GGE-biplot was employed to decompose the G × E interaction, to determine the yield and stability of the PVA hybrids across research environments. The principal component 1(PC1) axis explained 65.8%, while the PC2 explained 9% of the total variation jointly accounting for about 75% of the total variation (Figure 3). The GGE biplot for grain yield of the best 25 and the worst four PVA hybrids alongside the six checks evaluated at 13 locations under drought and low soil-N conditions, artificial *Striga* infestation and optimal growing conditions is shown in Figures 3 and 4. In the entry/tester view (mean vs. stability) of the GGE biplot (Figure 3), the double-arrowed line separated entries with below-average means from those with above-average means. The average yield of a cultivar is approximated by the projections of their markers on the average-tester axis while the stability of a hybrid is measured by its projection onto the double-arrow line (average-tester coordinate (y) axis). Hybrids 3 (TZEIOR 4 × TZEIOR 158), 21 (TZEIOR 119 × TZEIOR 158) and 4 (TZEIOR 4 × TZEIOR 167) were the highest yielding and the most stable hybrids across the 13 research environments while hybrids 1 (TZEIOR 2 × TZEIOR 6), 28 (TZEIOR 164 × TZEIOR 165), and 26 (TZEIOR 157 × TZEIOR 158) were the lowest yielding and most unstable across test locations. In the polygon view (Figure 4), the vertex hybrid in each sector indicated the highest yielding hybrid in the locations that fell within the sector. Therefore, entry 3 (TZEIOR 4 × TZEIOR 158) was the highest yielding hybrid at E1 (Ikenne under optimal conditions in 2016), E2 (Kadawa, under optimal conditions in 2016), E4 (Mokwa, under low-N in 2016), E6 (Mokwa, under *Striga* infestation in 2016), E7 (Ikenne, under optimal conditions in 2017), E8 (Abuja, under optimal conditions in 2017) and E11 (Mokwa, under *Strig*a infestation in 2017); hybrid 17 (TZEIOR 73 × TZEIOR 158) was the top ranking hybrid at E3 (Mokwa, under optimal growing conditions in 2016), E10 (Mokwa, under low-N in 2017) and E12 (Abuja, under *Striga* infestation in 2017); while entry 5 (TZEIOR 6 × TZEIOR 73) was the top ranking hybrid at E5 (Ikenne, under drought condition) and E9 (Bagauda, under optimal conditions).

Table 2. Mean squares from the ANOVA of grain yield and other agronomic traits of 190 early maturing pro-vitamin A hybrids and 6 hybrid checks evaluated across three stress conditions (drought, low-N and under *Striga* infestation) in 2016 and 2017 in Nigeria.

Source	DF	Yield	DA	DS	ASI	PLHT	EHT	RL	SL	HC	EASP	EROT	EPP
Block (Rep*E)	182	1,956,126 **	11.22 **	19.11 **	7.51 **	666.04 **	211.55 **	1.26 **	3.28 **	1.65 **	1.62 **	3.31 **	0.12 **
Rep (E)	7	5,151,433 **	98.21 **	82.22 **	14.59 *	4257.66 **	1820.75 **	2.56 **	8.94 **	7.18 **	13.98 **	185.93 **	0.56 **
Environment (E)	6	454,529,301 **	2301.2 **	6066.04 **	2751.35 **	186,377.2 **	32,883.84 **	79.34 **	327.16 **	503.83 **	359.34 **	564.97 **	21.07 **
Genotype (G)	189	2,760,720 **	24.97 **	26.25 **	7.59 **	671.91 **	211.10 **	1.54 **	4.20 **	1.79 **	2.72 **	2.57 **	0.14 **
GCA	19	8,183,523 **	120.13 **	106.35 **	17.17 **	1994.47 **	445.45 **	5.75 **	18.19 **	6.17 **	5.77 **	6.94 **	0.18 **
SCA	170	2,392,470 **	17.35 **	19.968 **	6.81	615.25 **	210.90 **	1.29 **	2.89 **	1.57 **	2.69 **	2.58 **	0.15 **
G × E	1134	1,250,691 **	6.55 **	9.95 **	7.12 **	387.08 **	140.58 **	1.33 **	3.32 **	1.06 **	1.42 **	2.24 **	0.11 **
GCA*E	114	3,570,297 **	6.94	16.93 **	11.25 **	648.03 **	218.14 **	2.87 **	7.22 **	15.25 **	19.53 **	4.48 **	0.22 **
SCA*E	1020	1,258,890 **	7.2 **	10.98 **	7.01 **	416.46 **	149.06 **	1.24 **	3.01 **	6.60 **	8.48 **	2.18 **	0.09 *
Error	1140	525,278	5.44	7.36	5.50	311.66	113.68	0.86	2.02	0.71	0.873	1.60	0.08

Source	DF	PASP	STGR	Source	DF	RAT1	RAT 2	STRC0_1	STRC0_2
Block (Rep*E)	104	1.79 **	1.65 **	Block (Rep*E)	78	2.54 **	2.71 **	3.33 *	2.86 **
Rep (E)	4	7.18 **	15.29 **	Rep(E)	3	2.98 **	2.91 **	7.730 *	7.45 **
Environment (E)	3	322.12 **	423.02 **	Environment (E)	2	107.59 **	136.59 **	252.85 **	165.20 **
Genotype (G)	189	1.62 **	1.10 **	Genotype (G)	189	3.22 **	3.78 **	3.04 *	2.75 **
GCA	19	2.35 **	2.70 **	GCA	19	1.85 **	2.11 **	2.76 **	2.41 **
SCA	170	1.94 **	1.30 **	SCA	170	2.51 **	2.45 **	2.54	2.01
G × E	567	1.17 **	0.93 **	G × E	378	2.08 **	2.05 **	2.54	2.01
GCA*E	57	2.66 **	2.24 **	GCA*E	114	9.6633	8.6745	2.2	1.71
SCA*E	510	1.25 **	0.98 **	SCA*E	1020	1.3976	1.4087	2.63	2.08
Error	652	0.749654	0.67793	Error	489	0.778814	0.734521	2.407927	1.94947

*, ** indicate significance at 0.05 and 0.01 probability levels, respectively; Rep is replication; ASI is Anthesis-silking interval; STGR is stay-green characteristic.

Table 3. Mean squares from the ANOVA of grain yield and other agronomic traits of 190 early maturing pro-vitamin A hybrids and 6 hybrid checks evaluated under optimal conditions and across stress and non-stress conditions in 2016 and 2017 in Nigeria.

Source of Variation	Df	Grain Yield, (kg/ha)	Days to Anthesis	Days to 50% Silk	ASI	Plant Height (cm)	Ear Height (cm)	Husk Cover	Ear Aspect	Ears/Plant
Optimal Environments										
Block (Rep*E)	156	2,572,781 **	6.8 **	7.6 **	0.5	429.5 **	218.6 **	0.8 **	0.8 **	0.02 **
Rep (E)	6	33,916,855 **	55.2 **	58.9 **	1.1 *	4806.1 **	1888.7 **	10.5 **	9.8 **	0.08 **
Environment (E)	5	1,602,594,010 **	770.7 **	451.2 **	213.9 **	212,914.3 **	46,556.3 **	1323.5 **	390.3 **	7.06 **
Genotype (G)	189	14,131,291 **	23.9 **	30.3 **	2.1 **	1213.9 **	432.5 **	4.3 **	6.2 **	0.11 **
GCA	19	9,372,017 *	99.1 **	136.1 **	12.5 **	3921.6 **	1238.4 **	4.2	4.7 **	0.21 **
SCA	170	15,600,485 **	18.1 **	21.8 **	1.0	1013.1 *	385.2 **	4.6	6.8 **	0.11 **
G × E	945	1,637,692 **	2.9 **	3.6 **	0.8 **	305.6 **	132.0 **	1.1 **	0.7 **	0.03 **
GCA*E	95	2,749,325 **	2.0	2.2	1.4 **	580.3 **	264.4 **	1.6 **	0.9 **	0.05 **
SCA*E	850	1,758,594 **	3.7 **	4.2 **	0.7 **	328.7 **	136.2 **	1.2 **	0.8 **	0.03 **
Error	1014	946,352	1.7	2	0.4	185.5	100	0.6	0.5	0.01
Across environments										
Block (Rep*E)	338	2,255,103 **	8.7 **	13.4 **	3.7 **	549.4 **	207.3 **	1.2 **	1.3 **	0.05 **
Rep (E)	13	19,213,986 **	83.9 **	74.8 **	9.6 **	4697.8 **	2009.8 **	9.3 **	13.2 **	0.36 **
Environment (E)	12	1,851,902,351 **	1794.4 **	4904.3 **	2011.6 **	291,143.7 **	62,019.4 **	1028.5 **	474.1 **	23.56 **
Genotype (G)	189	12,220,910 **	44.7 **	50.9 **	5.4 **	1505.3 **	466.1 **	4.4 **	7.0 **	0.15 **
GCA	19	12,793,056.2 **	213.2 **	230.4 **	25.8 **	4694.6 **	1201.0 **	8.7 **	5.9 **	0.18 *
SCA	170	12,928,333.76 **	30.6 **	35.9 **	3.5 **	1243.8	411.8 **	4.3 *	7.6 **	0.17 **
G × E	2268	1,667,261 **	4.7 **	7.0 **	3.6 **	356.8 **	143.7 **	1.1 **	1.1 **	0.04 **
GCA*E	228	3,315,645 **	5.2 **	10.4 **	6.5 **	628.6 **	256.6 **	2.1 **	2.3 **	0.11 **
SCA*E	2040	1,729,213 **	5.3 **	7.8 **	3.5 **	381.5 **	148.9 **	1.2 **	1.1 **	0.04 **
Error	2197	744,268	3.3	4.8	2.6	249.7	107.4	0.7	0.7	0.02

*, ** indicate significance at 0.05 and 0.01 probability levels, respectively; Rep is replication; ASI is Anthesis-silking interval.

Table 4. General combining ability (GCA) effects for grain yield and other agronomic traits of the 20 early maturing pro-vitamin A inbred lines crossed in diallel fashion and evaluated under stress, non-stress and across both conditions between 2016 and 2017 in Nigeria.

INBRED	Grain Yield (Kg/ha)			Days to Silk			Days to Anthesis			Plant Aspect		
	STR	NON-STR	ACR	STR	NON-STR	ACR	STR	N0N-STR	ACR	STR	NON-STR	ACR
TZEIOR 2	−144.15	53.49	−21.24	0.61 *	1.15 **	0.77 **	0.56 *	0.25 **	0.63 **	−0.01	−0.14 *	−0.11 *
TZEIOR 4	−3.43	399.83 **	208.41	0.33	0.50 **	0.45 **	0.26	0.15 *	0.34 **	0.04	−0.15 *	−0.11
TZEIOR 6	−0.98	78.95	41.75	0.41	1.05 **	0.67 **	0.3	0.14	0.65 **	0.18	−0.12 *	−0.058
TZEIOR 30	−263.31 **	128.09	−55.24	−0.08	−0.68 **	−0.39 **	0.06	−0.16 *	−0.34 **	−0.09	−0.14 *	−0.071
TZEIOR 52	303.48 **	58.22	180.15	0.14	0.38 **	0.40 **	0.61 **	−0.06	0.55 **	−0.16	−0.1	−0.078
TZEIOR 62	−35.66	−151.5	−139.77	0.29	0.27	0.41 **	0.61 **	−0.31 **	0.62 **	−0.04	0.02	0.018
TZEIOR 68	−75.14	16.99	−31.43	0.34	0.001	0.1	0.48 *	−0.21 **	0.25	0.11	0.13 *	0.19
TZEIOR 73	−73.8	−34.36	−45.74	0.88 **	0.29 *	0.63 **	1.11 **	−0.26 **	0.73 **	−0.04	0.15 **	0.079
TZEIOR 79	−375.45 **	−442.41 **	−501.82 **	0.72 *	0.32 *	0.66 **	0.74 **	−0.17 *	0.70 **	0.03	0.21 **	0.19 **
TZEIOR 117	−4.01	−25.38	1.91	−1.11 **	−1.49 **	−1.33 **	−1.03 **	−0.30 **	−1.05 **	0.24 *	0.29 **	0.22 **
TZEIOR 119	175.29	−79.74	80.72	−1.09 **	−1.41 **	−1.36 **	−1.28 **	−0.12	−1.20 **	−0.1	0.09	0.001
TZEIOR 124	−249.35 **	−392.22 *	−331.84 **	−0.14	−0.23	−0.1	−0.18	0.03	−0.13	0.002	0.09	0.041
TZEIOR 125	−155.19	−293.39	−244.96 *	−0.48	−0.60 **	−0.61 **	−0.55 *	−0.04	−0.54 **	0.05	0.03	0.01
TZEIOR 157	71.02	50.04	18.67	0.71 *	0.56 **	0.66 **	0.44 *	0.34 **	0.40 **	0.06	−0.02	0.001
TZEIOR 158	156.7	−73.8	32.2	0.62 *	1.19 **	0.88 **	0.55 *	0.55 **	0.55 **	−0.02	0.03	−0.004
TZEIOR 163	228.21 *	−12.9	92.57	0.19	0.60 **	0.53 **	−0.24	0.27 **	0.19	−0.16	−0.01	−0.039
TZEIOR 164	144.26	181.9	234.097 *	−0.42	−0.37 *	−0.51 **	−0.78 **	0.13	−0.66 **	0.11	0.03	0.033
TZEIOR 165	114.71	71.6	123.34	−0.5	−0.37 *	−0.46 **	−0.91 **	0.18 *	−0.68 **	0.14	0.1	0.13 *
TZEI 25	219.01 *	151.53	222.68 *	−0.11	0.03	−0.2	0.12	−0.1	−0.08	−0.08	−0.28 **	−0.28 **
TZEI 129	−32.2	315.08 *	135.53	−1.33 **	−1.21 **	−1.21 **	−0.88 **	−0.28 **	−0.95 **	−0.28 *	−0.19 **	−0.17 **
TZEIOR 2	0.15	−0.02	0.055	−0.03	0.013	−0.012	0.03	0.02	0.36 **	0.34 **	5.80 *	7.44 **
TZEIOR 4	0.001	−0.14	−0.084	−0.02	−0.005	−0.018	0.34 *	0.09	0.17	0.22	8.04 **	8.29 **
TZEIOR 6	0.11	−0.05	0.002	−0.01	0.004	−0.0001	0.16	0.07	−0.21	−0.11	7.71 **	8.23 **
TZEIOR 30	0.24 **	−0.14	0.098	−0.04	0.015	−0.021	−0.33 *	0.16	0.63 **	0.61 **	5.11 *	5.64 *
TZEIOR 52	−0.26 **	−0.17 *	−0.22 **	0.04	0.009	0.03 *	−0.41 **	0.04	−0.91 **	−0.95 **	−4.66	−5.56 *
TZEIOR 62	−0.02	−0.09	−0.035	0.01	0.059 **	0.04 **	0	0.13	0.06	0.04	4.35	4.80 *
TZEIOR 68	0.01	−0.07	0.007	0.01	0.022	0.029	0.07	0.23	0.11	0.14	3.14	4.14
TZEIOR 73	0.03	−0.15	−0.091	−0.003	0.037 **	0.015	−0.13	0.2	0.42 **	0.53 **	5.20 *	4.74 *
TZEIOR 79	0.27 **	0.12	0.23 **	0.01	0.053 **	0.023	−0.23	0.16	0.41 **	0.52 **	2.58	2.09

TZEIOR 117	−0.02	0.15	0.039	−0.01	0.011	0.002	0.35 **	−0.09	0.32 *	0.36 **	−5.34 *	−5.82 *
TZEIOR 119	−0.21 *	0.06	−0.104	0.004	−0.003	0.001	0.02	−0.2	−0.06	−0.06	1.36	1.72
TZEIOR 124	0.19 *	0.20 *	0.18 **	−0.05 *	−0.032 *	−0.04 **	−0.05	−0.13	0.66 **	0.66 **	3.62	3
TZEIOR 125	0.15	0.12	0.15 *	−0.02	−0.025	−0.028	0.03	−0.18	0.29 *	0.25 *	−0.3	−0.58
TZEIOR 157	0.01	0.17 *	0.126	−0.01	−0.048 **	−0.032	−0.07	−0.16	−0.53 **	−0.71 **	−4.33	−4.4
TZEIOR 158	−0.1	0.31 **	0.125	−0.003	−0.048 **	−0.03 *	0.12	−0.14	−0.50 **	−0.53 **	−6.40 *	−7.24 **
TZEIOR 163	−0.18 *	0.13	−0.004	0.03	−0.049 **	−0.016	0.13	−0.17	−0.40 **	−0.52 **	−3.39	−2.78
TZEIOR 164	−0.15	−0.08	−0.14 *	0.06 *	−0.008	0.007	0.25	0.16	−0.51 **	−0.43 **	−8.22 **	−6.66 **
TZEIOR 165	−0.11	−0.01	−0.109	0.02	−0.022	0.04 **	0.35 **	0.05	−0.23	−0.24	−5.79 *	−7.78 **
TZEI 25	−0.1	−0.1	−0.129	−0.001	−0.008	−0.003	−0.11	−0.31 *	−0.33 **	−0.41 **	−7.45 **	−7.38 **
TZEI 129	−0.02	−0.24 **	−0.106	0.01	0.025	0.01	−0.52 **	0.05	0.25 *	0.30 *	−1.03	−1.9

*, ** is the significance at 0.05 and 0.01 probability levels, respectively.

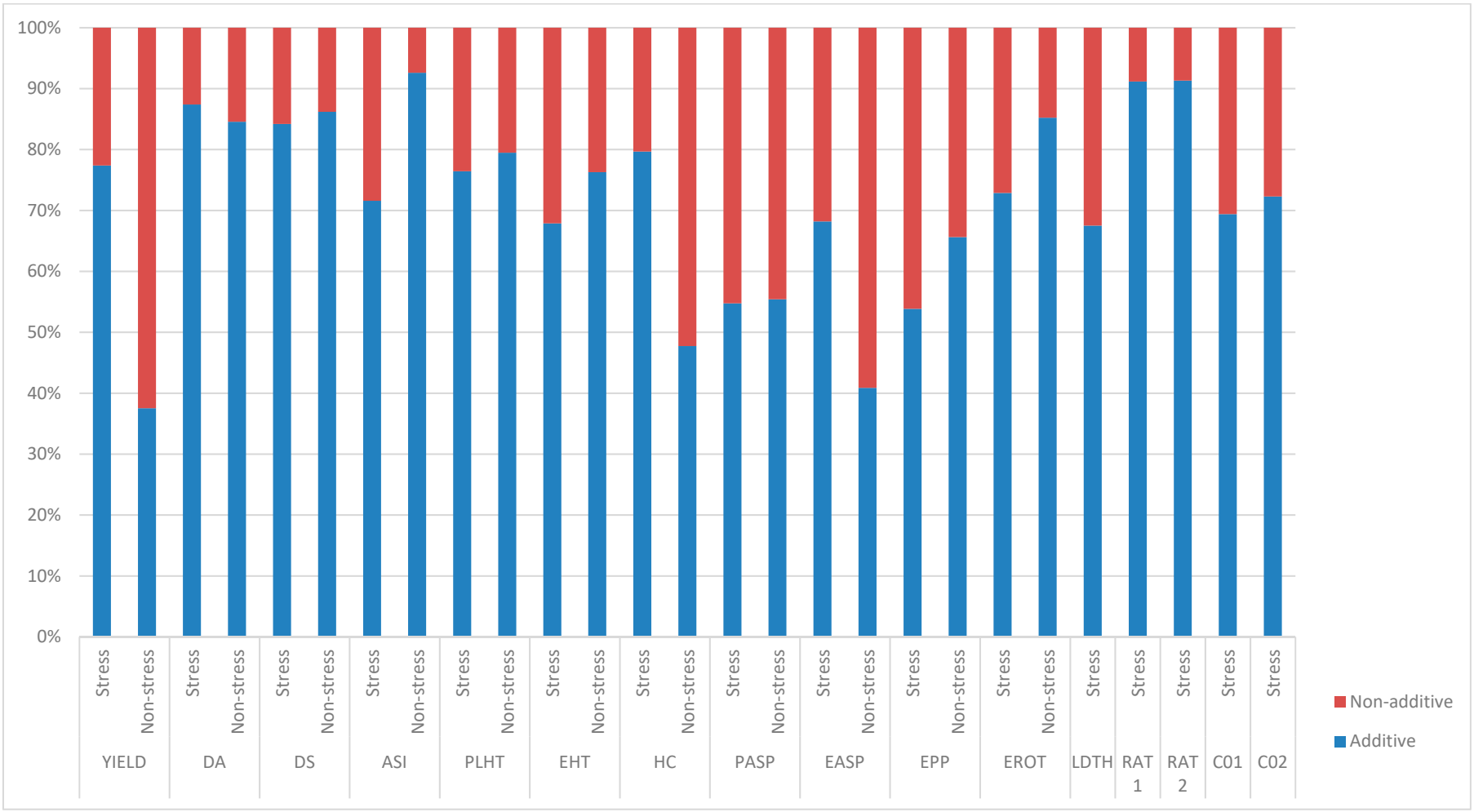


Figure 2. Proportion of additive (lower bar) and non-additive (upper bar) genetic variance for grain yield and other agronomic traits under stress, non-stress, and across test environments in a diallel among 20 early pro-vitamin A inbred lines.

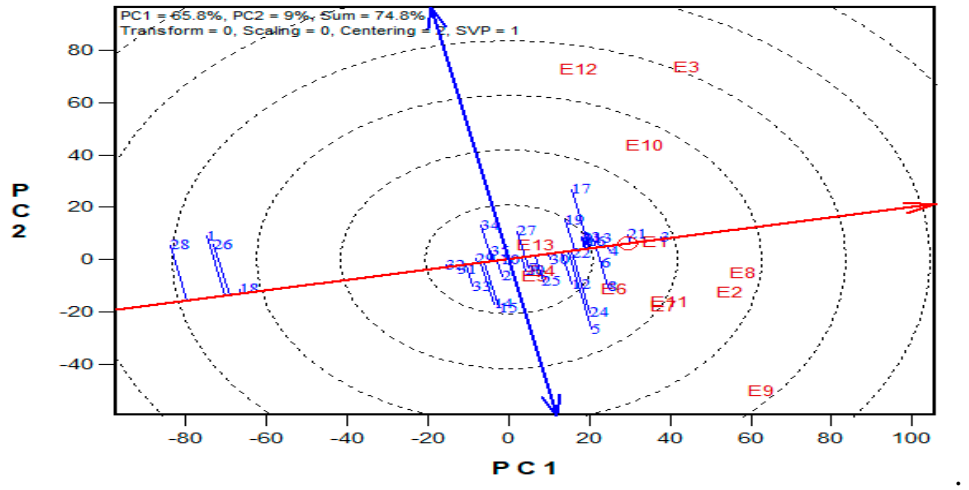
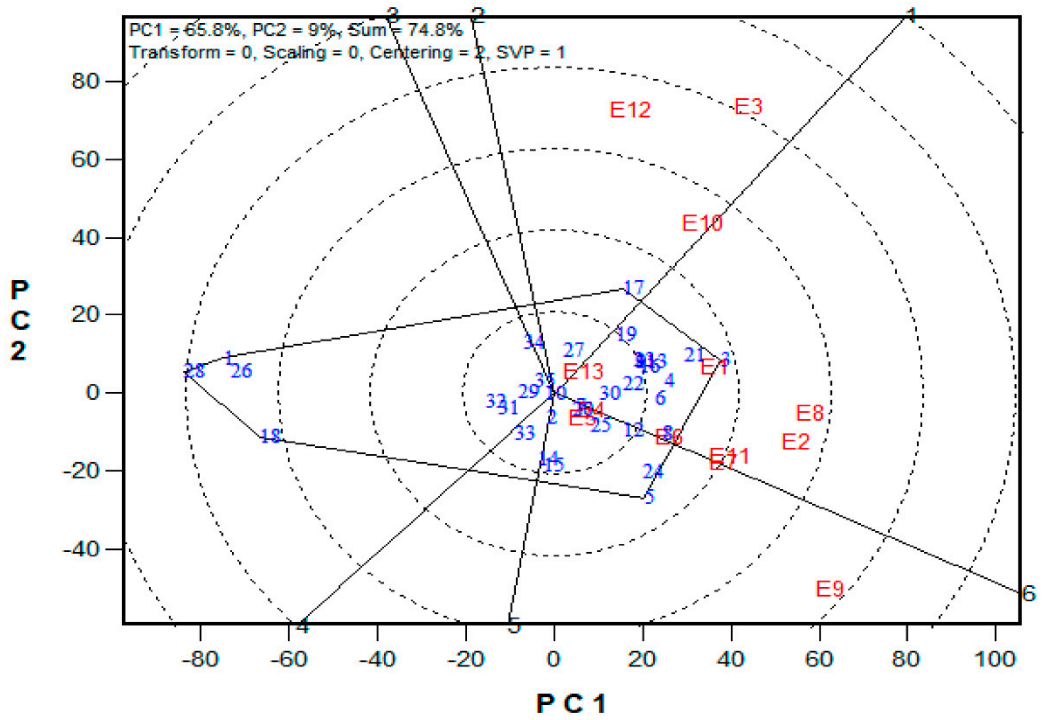


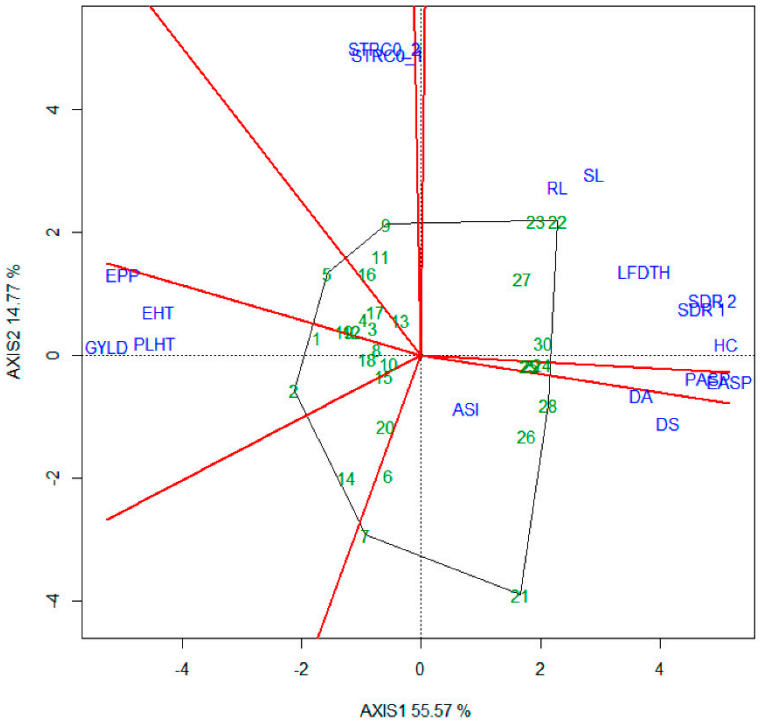
Figure 3. A Mean vs. Stability view based on a genotype x environment yield data of 29 early maturing pro-vitamin A hybrids and 6 checks evaluated in 13 environments under drought, low-N, Striga infestation and optimal conditions between 2016 and 2017. The biplot was based on environment-focused singular value partitioning ('SVP = 1) and is therefore appropriate for visualizing the relationships among genotypes. Principal component (PC)1 and PC 2 explained 51.1% of yield variation.

The genotype × trait (G × T) biplot (Figure 5) shows the performance of the genotypes with respect to different agronomic traits across the stress conditions. Genotypes in the same sector with traits are associated with such traits, while genotype(s) at the vertex of a sector was the best for the trait(s) in the sector and the worst for traits in the opposing sector. Result of relationship among genotypes and genotypes by trait interaction was substantiated by standardizing means of the evaluated hybrids. Under stress, the G × T biplot accounted for 70.34% of the total variation, axis 1(PC 1) = 55.57%, axis 2 (PC 2) =14.77%. Hybrid 1 (TZEIOR 119 × TZEI 158) and hybrid 2 (TZEIOR 119 × TZEIOR 25) were outstanding for grain yield, number of ears per plant, plant, and ear heights. Additionally, hybrid 9 (TZEIOR 6 × TZEIOR 163) recorded the highest number of emerged *Striga* plants at 8 and 10 WAP. Hybrid 22 (TZEIOR 4 × TZEIOR 6) was associated with high *Striga* damage at 8 and 10 WAP, stem lodging, root lodging, leaf-death score, and husk cover. The vector view of genotype by trait (GT) biplot showing the relationships among traits (Figure 6) across stress conditions explained 70.34% of the total variation among the traits of the single-cross hybrids. In the display, the line that connects the trait to the biplot origin is called a trait vector. The cosine of the angle between any two vectors measured the correlation between the traits. The biplot display revealed a high positive and significant relationship among grain yield, number of ears per plant, plant and ear heights while these traits showed a negative relationship with plant and ear aspects, *Striga* damage at 8 and 10WAP, husk cover and leaf-death score. However, there was weak relationship between grain yield and number of emerged *Striga* plants at 8 and 10 WAP. The G × T biplot under optimal growing conditions (Figure 7) explained 64.76% of the total variation with axis PC 1 = 47.16%, PC 2 = 26.73%. Hybrid 1 (TZEIOR 4 × TZEIOR 158) was the best hybrid for grain yield and number of ears per plant under optimal conditions. Hybrid 12 (TZEIOR 4 × TZEIOR 157) was associated with high ratings for plant and ear heights while hybrid 21 (TZEIOR 4 × TZEIOR 6) was associated with high plant aspect, ear aspect and husk cover. The 'relationship among traits' biplot under optimal conditions (Figure 8) explained 64.8% of the observed variation for the evaluated hybrids. There was positive association among grain yield, ears per plant, plant height, and ear height. These traits showed a negative relationship with plant aspect, ear aspect, husk cover, number of days to 50% flowering and number of days to 50% silking.



E1	**IKOPT16**
E2	**KO16**
E3	**MOOPT16**
E4	**MOLN16**
E5	**IKDS16**
E6	**MOSTR16**
E7	**IKOPT17**
E8	**ABOPT17**
E9	**BG17**
E10	**MOLN17**
E11	**MOSTR17**
E12	**ABSTR17**
E13	**IKDS17**

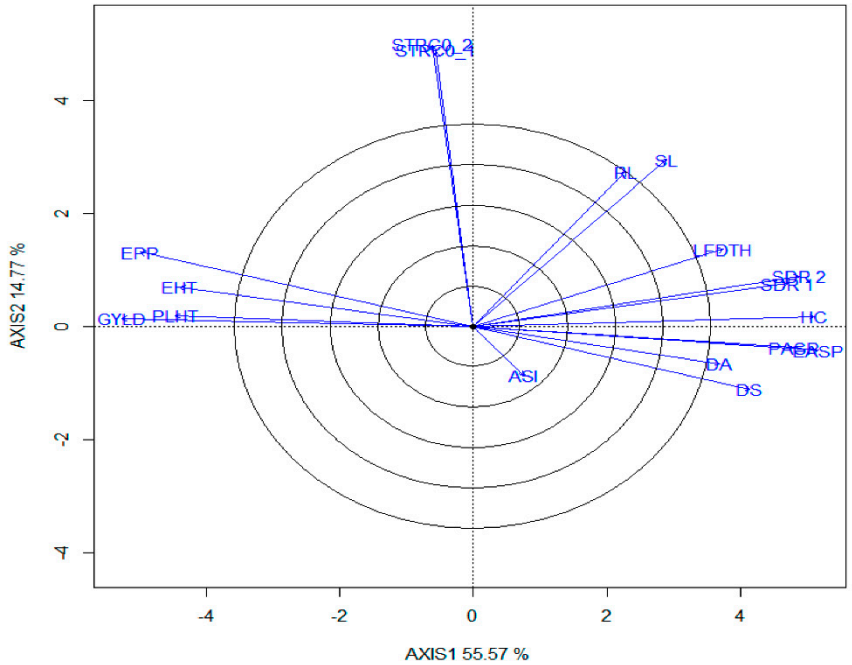
Figure 4. A Which-won where or which-is-best-at-what based on a genotype × environment yield data of 29 early maturing pro-vitamin A hybrids and 6 checks evaluated in 13 environments under drought, low-N, Striga infestation and optimal conditions between 2016 and 2017. The biplot was based on genotype-focused singular value partitioning ('SVP = 2) and is therefore appropriate for visualizing the relationships among environments. Principal component (PC) 1 and PC 2 explained 74.8% of yield variation.



Entry	Crosses
1	TZEIOR 119 x TZEIOR 158
2	TZEIOR 119 x TZEI 25
3	TZEIOR 52 x TZEIOR 163
4	TZEIOR 4 x TZEIOR 158
5	TZEIOR 62 x TZEIOR 157
6	TZEIOR 52 x TZEIOR 164
7	TZEIOR 157 x TZEI 25
8	TZEIOR 6 x TZEI 25
9	TZEIOR 6 x TZEIOR 163
10	TZEIOR 6 x TZEIOR 165
11	TZEIOR 119 x TZEIOR 164
12	TZEIOR 62 x TZEIOR 158
13	TZEIOR 119 x TZEIOR 165
14	TZEIOR 158 x TZEI 25
15	TZEIOR 117 x TZEI 25
16	TZEIOR 62 x TZEIOR 163
17	TZEIOR 125 x TZEI 25
18	TZEIOR 125 x TZEIOR 164
19	TZEIOR 117 x TZEIOR 157
20	TZEIOR 117 x TZEIOR 158
21	TZEIOR 157 x TZEIOR 158
22	TZEIOR 4 x TZEIOR 6
23	TZEIOR 62 x TZEIOR 73
24	TZEIOR 117 x TZEIOR 124
25	TZEIOR 73 x TZEIOR 79
26	TZEIOR 164 x TZEIOR 165
27	TZEIOR 119 x TZEIOR 124
28	TZEIOR 62 x TZEIOR 79
29	TZEIOR 117 x TZEIOR 125
30	TZEIOR 124 x TZEIOR 125

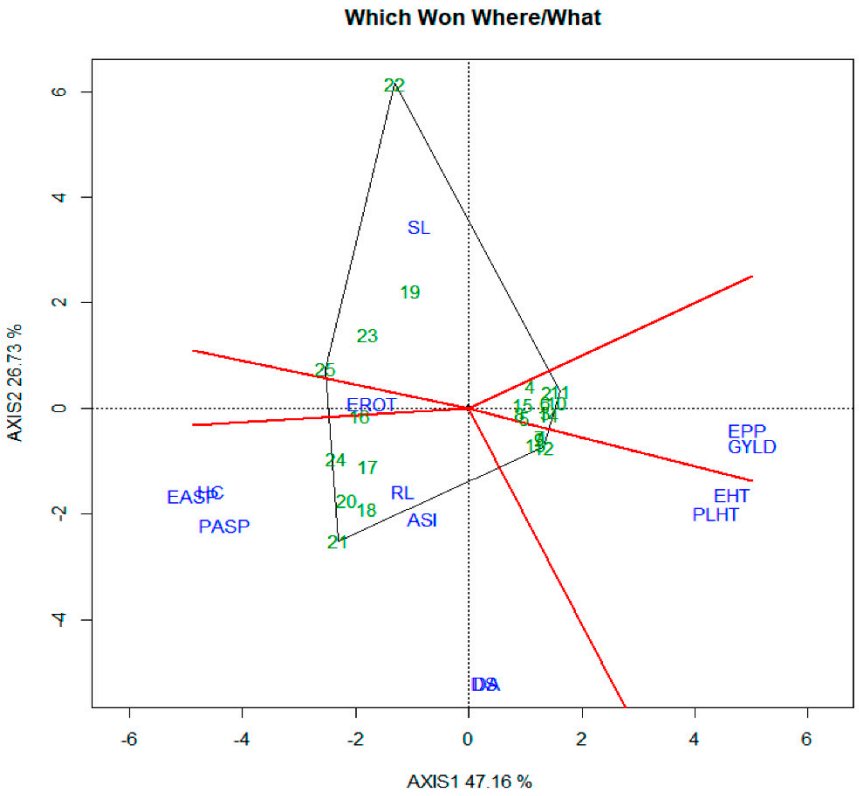
Trait	Code
Grain yield	GYLD
Plant height	PLHT
Ear height	EHT
Ears per plant	EPP
Ear aspect	EASP
Days to 50 % silking	DS
Days to 50% anthesis	DA
Anthesis-silking interval	ASI
Striga damage ratings at 8 WAP	SDR1
Striga damage ratings at 10 WAP	SDR2
Emerged *Striga* plants at 8WAP	ESP1
Emerged *Striga* plants at 10 WAP	ESP2
Root lodging	RL
Stalk lodging	SL
Husk cover	HC
Ear rot	EROT
Leaf death score	LFDTH

Figure 5. A polygon view of genotype by trait showing top 20 hybrids and the least 10 hybrids among the 196 testcrosses evaluated under Striga infestation at Mokwa and Abuja, drought stress at Ikenne and low N at Mokwa and Ile-Ife, Nigeria in 2016 and 2017. The biplot was based on genotype-focused singular value partitioning ('SVP = 2). Principal component (axis 1) and axis 2 for model 2 explained 70.34% of the variation among traits.



Trait	Code
Grain yield	GYLD
Plant height	PLHT
Ear height	EHT
Ears per plant	EPP
Ear aspect	EASP
Days to 50 % silking	DS
Days to 50% anthesis	DA
Anthesis-silking interval	ASI
Striga damage ratings at 8 WAP	SDR1
Striga damage ratings at 10 WAP	SDR2
Emerged *Striga* plants at 8WAP	ESP1
Emerged *Striga* plants at 10 WAP	ESP2
Root lodging	RL
Stalk lodging	SL
Husk cover	HC
Ear rot	EROT
Leaf death score	LFDTH

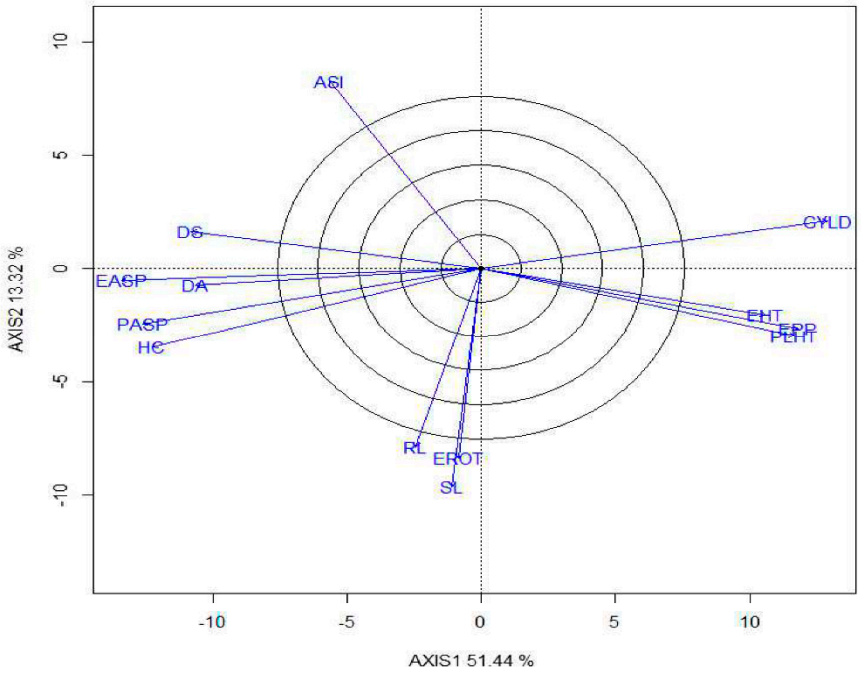
Figure 6. A vector view of the genotype-by-trait biplot showing interrelationships among all traits of 196 early maturing pro-vitamin A hybrid maize evaluated under Striga infestation at Mokwa in 2016 and 2017 and at Abuja in 2017. The biplot was based on genotype-focused singular value partitioning ('SVP = 2) and is therefore appropriate for visualizing the relationships among traits. Principal component (axis 1) and axis 2 for model 2 explained 70.34% of the variation among traits.



Entry	Pedigree
1	TZEIOR 4 x TZEIOR 158
2	TZEIOR 4 x TZEIOR 165
3	TZEIOR 4 x TZEIOR 164
4	TZEIOR 62 x TZEIOR 165
5	TZEIOR 6 x TZEIOR 165
6	TZEIOR 117 x TZEIOR 163
7	TZEIOR 2 x TZEIOR 157
8	TZEIOR 2 x TZEIOR 165
9	TZEIOR 6 x TZEIOR 163
10	TZEIOR 62 x TZEIOR 158
11	TZEIOR 62 x TZEIOR 163
12	TZEIOR 4 x TZEIOR 157
13	TZEIOR 2 x TZEIOR 163
14	TZEIOR 68 x TZEIOR 163
15	TZEIOR 2 x TZEIOR 164
16	TZEIOR 117 x TZEIOR 125
17	TZEIOR 117 x TZEIOR 124
18	TZEIOR 157 x TZEIOR 158
19	TZEIOR 124 x TZEIOR 125
20	TZEIOR 158 x TZEIOR 163
21	TZEIOR 4 x TZEIOR 6
22	TZEIOR 62 x TZEIOR 68
23	TZEIOR 2 x TZEIOR 6
24	TZEIOR 164 x TZEIOR 165
25	TZEIOR 157 x TZEIOR 163

Trait	Code
Grain yield	GYLD
Plant height	PLHT
Ear height	EHT
Plant aspect	PASP
Ear aspect	EASP
Days to 50 % silking	DS
Days to 50% anthesis	DA
Anthesis-silking interval	ASI
Ears per plant	EPP
Root lodging	RL
Stalk lodging	SL
Husk cover	HC
Ear rot	EROT

Figure 7. A polygon view of genotype by trait showing top 15 hybrids and the least 10 hybrids among the 196 testcrosses evaluated under optimal conditions at Bagauda, Kadawa, Abuja, Ikenne and Mokwa, Nigeria in 2016 and 2017. The biplot was based on genotype-focused singular value partitioning ('SVP = 2). Principal component (axis 1) and axis 2 for model 2 explained 64.76% of the variation among traits.



Trait	Code
Grain yield	GYLD
Plant height	PLHT
Ear height	EHT
Plant aspect	PASP
Ear aspect	EASP
Days to 50 % silking	DS
Days to 50% anthesis	DA
Anthesis-silking interval	ASI
Ears per plant	EPP
Root lodging	RL
Stalk lodging	SL
Husk cover	HC
Ear rot	EROT

Figure 8. A vector view of the genotype-by-trait biplot showing interrelationships among all traits of 190 early maturing pro-vitamin A hybrid maize evaluated under optimal conditions at Bagauda, Kadawa, Abuja, Ikenne and Mokwa, Nigeria in 2016 and 2017. The biplot was based on genotype-focused singular value partitioning ('SVP = 2) and is therefore appropriate for visualizing the relationships among traits. Principal component (axis 1) and axis 2 for model 2 explained 64.76% of the variation among traits.

4. Discussion

The significant genotype mean squares observed for all traits measured under stress, optimal and across research conditions were indicative of the existence of high genetic variability for grain yield in the genotypes which can be exploited for accelerated genetic gains in the improvement of these traits. Also, the significant mean squares observed for the environment suggested the uniqueness of the capacity of the test environments to discriminate among the genotypes under stress and non-stress conditions. The significant GEI obtained for most measured traits in stress, non-stress and across environments revealed differential performances of the genotypes under contrasting environmental conditions, thus the need for identification and selection of outstanding and stable genotypes across environments [25,26]. Additionally, the significant GEI observed for grain yield and most other traits confirmed the importance of multi-environment testing of hybrids before recommendations for release and commercialization could be made [43]. The significant GCA × E interaction effect detected for most measured traits suggested significant differences in the combining ability of the PVA inbred lines under varying environmental conditions, suggesting the need for testing the lines in contrasting environments for identification of those with stable performance for the development of multiple stress tolerant hybrids [43].

The significant GCA and SCA mean squares obtained for grain yield and most other measured traits in the test environments indicated that both additive and non-additive gene actions were important in the inheritance of the measured traits of the PVA genotypes evaluated in the present study. Variations in the GCA mean squares of the inbred lines further suggested the availability of genetic variability among the inbreds under each environment which suggests a great potential for classifying the inbred lines into heterotic groups as well as identifying lines with high general combining abilities which could be useful as testers. Additionally, the preponderance of GCA over SCA effects for grain yield and other measured traits in both stress and non-stress environments, except for grain yield across multiple stress conditions revealed the relative importance of additive gene action to non-additive gene action in the inheritance of grain yield and other measured traits of the PVA inbred lines. This was an indication that most of the measured traits could be improved through recurrent selection methods such as the full-sib, half-sib. This result is consistent with the findings of [43]. Significant GCA for grain yield and most other traits facilitated the identification of potentially discriminating inbred testers as well as candidate parental inbreds for hybrid development. Additionally, this result indicated that most of the traits could be improved through population improvement methods such as the S_1 family recurrent selection, half-sib and full-sib recurrent selection methods. Additionally, the presence of significant SCA effects indicated that the backcrossing method could be employed for hybrid formation, and development of synthetics. The significant SCA × E interaction for grain yield and most measured traits signified differential performance of the hybrids under contrasting environmental conditions, indicating the need for selection of hybrids best adapted to each environment. However, the insignificant SCA × E for number of emerged *Striga* plants implied consistency in hybrid performance across *Striga*-infested environments. The results further lend credence to the approach of evaluating hybrids under contrasting conditions to enable identification of superior hybrids for each and across research environments. The HGCAMT method used in this study classified the early PVA inbred lines into three heterotic groups, effectively placing the parental lines of high yielding hybrids into opposing heterotic groups. However, the parental inbreds of the low-yielding hybrids were not necessarily placed in the same heterotic group.

A prime objective of this study was to identify high yielding and stable PVA hybrids across stress and non-stress conditions. The present study showed that some PVA hybrids out-yield the normal endosperm hybrids under stress and non-stress conditions. The GGE biplot identified TZEIOR 4 × TZEIOR 158 as the most stable and highest yielding PVA

hybrid in the present study, out yielding the PVA commercial hybrid check, TZEI 124 × TZEI 25 by 25.6% across research conditions. This implied that the PVA hybrid has the potential to replace this commercial check in the public domain. It is important to note that TZEIOR 119 × TZEIOR 158 and TZEIOR 4 × TZEIOR 165 were also high yielding and stable across research environments. Following an extensive evaluation for consistency in performance, these PVA hybrids could be commercialized to combat malnutrition and food insecurity in SSA.

An inbred parent with significant and positive GCA effects for a target trait is a good combiner for such trait and has a high probability of passing the characteristics to its progeny in a cross. Genotypes with positive and significant GCA effects for grain yield and other desirable traits could therefore be used for the development of heterotic populations as well as high yielding synthetics and hybrids ([9]. Significant and positive GCA effects were observed for the grain yield of TZEIOR 52, TZEI 25 and TZEIOR 163 under multiple stresses, while TZEIOR 4 and TZEI 129 possessed positive and significant GCA effects under optimal conditions, and TZEIOR 164 and TZEI 25 across research conditions. This indicated that these inbred lines possessed beneficial alleles for grain yield and would contribute high grain yield to their progenies under the contrasting environmental conditions. Contrarily, significant and negative GCA effects were recorded for *Striga* damage and number of emerged *Striga* plants at 8 and 10 WAP for TZEIOR 164 and TZEI 25, implying that these inbred lines are invaluable resources for *Striga* resistance/tolerance alleles for improving tropical maize germplasm for multiple stress tolerance.

Fan et al., [40] reported that an efficient heterotic grouping method should classify inbred lines into groups which allow inter-heterotic group crosses to display higher heterosis than intra-heterotic groups. Badu Apraku et al., [43] compared different heterotic grouping methods and concluded that HGCAMT was the best method for grouping early QPM inbreds into heterotic groups with breeding efficiency of 82%. In the present study, the HGCAMT method placed the parental lines of the high yielding PVA hybrids into opposing heterotic groups further confirming the effectiveness of the grouping method at placing parental inbred lines into heterotic groups to maximize heterosis from planned crosses. Despite the effectiveness of the HGCAMT method in classifying the inbred lines into heterotic groups in the present study, it would be desirable to use molecular markers and genomic selection to confirm the heterotic groupings of the 20 early inbred lines.

Information on inter-trait relationships among grain yield and other agronomic traits will enhance the efficiency of breeding programs using appropriate traits as selection criteria under stress and non-stress conditions [17]. The results of the G × T biplot analysis under multiple stresses revealed that ASI had a short trait vector implying that it is not important in evaluating early maturing maize genotypes for stress tolerance. The small acute angle observed between grain yield and ears per plant as well as plant and ear heights indicated the existence of significant positive correlations between grain yield and these traits suggesting that they could serve as important selection criteria for improved grain yield across multiple stresses. Additionally, days to anthesis and silking, husk cover, *Striga* damage at 8 and 10 WAP and the stay green characteristic which had angles near 180^0 were negatively correlated with yield, an indication that these traits had direct or indirect effects on yield across stress environments. This result is consistent with the findings of [38,44,45]. The significant and positive correlations between grain yield and ears per plant, ear and plant heights implied that selection for improvement in these traits under optimal growing conditions will result in improved grain yield. Also, the significant negative correlation between grain yield and plant aspect, ear aspect, days to silking, and anthesis as well as husk cover in non-stress environments are indications that these traits are reliable selection indices for yield improvement in optimal conditions. TZEIOR 119 × TZEIOR 158 and TZEIOR 4 × TZEIOR 158 identified as high yielding and stable hybrids by both GGE and the G × T biplot analyses should be further tested for

consistency in performance and released for commercialization to combat malnutrition and food insecurity in SSA.

5. Conclusions

The significant mean squares obtained for genotypes for grain yield and other measured traits confirmed the availability of genetic variability for multiple stress tolerance among the early maturing maize. The significant GCA and SCA mean squares observed in the present study implied that both additive and non-additive gene actions modulated the inheritance of grain yield and other measured traits across the multiple stresses and optimal management conditions, however, additive gene action was more important. TZEI 25 and TZEIOR 164 were identified as inbred testers for groups 2 and 3, respectively. Plant and ear heights, ears per plant, plant and ear aspects were identified as invaluable selection criteria for yield improvement in both stress and non-stress management conditions. TZEIOR 4 × TZEIOR 158 and TZEIOR 119 × TZEIOR 158 were outstanding in terms of grain yield and stability and should be extensively tested and commercialized to combat malnutrition and food insecurity in SSA.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/agronomy11071371/s1, Table S1: Mean squares showing grain yield and other agronomic traits of 196 early maturing pro-vitamin A hybrids evaluated under Striga Infestation both in 2016 and 2017 in Nigeria, Table S2: Mean squares showing grain yield and other agronomic traits of 196 early maturing pro-vitamin A hybrids evaluated under Optimal conditions both in 2016 and 2017 in Nigeria.

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